UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

PC 059102

SUBJECT: PP#0F2423 and FAP#0H5277. Chlorpyrifos-methyl on Grains. Evaluation of analytical methods and residue data.

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THRU: Charles L. Trichilo, Chief
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Dow Chemical Company proposes the establishment of tolerances for combined residues of the insecticide chlorpyrifos-methyl (0,0-dimethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate) and its metabolite 3,5,6-trichloro-2-pyridinol in or on the following commodities:

Grains of barley, corn, oats, rice sorghum and wheat	6.0 ppm
Fat of cattle, goats and sheep	0.2 ppm
Meat of cattle, goats and sheep	0.1 ppm
Meat byproducts of cattle, goats and sheep	1.0 ppm
Fat of hogs and horses	0.3 ppm
Meat of hogs and horses	0.1 ppm
Meat byproducts of hogs and horses	0.5 ppm
Milk, whole	0.02 ppm
Milk, fat	0.1 ppm
Eggs	0.05 ppm
Poultry meat, fat and meat byproducts	0.05 ppm

Food additive tolerances are proposed for the following:

Corn oil	160 ppm
Milling fractions (except flour) of barley, corn, oats, sorghum and wheat	20 ppm
Milling fractions of rice	30 ppm

No tolerances have been established for chlorpyrifos-methyl to date and no other petitions are pending. This chemical is very similar to chlorpyrifos.

Conclusions

1a. A metabolism/degradation study on a stored grain is needed. Special attention should be given to the amount and nature of any conjugates of metabolites of chlorpyrifos-methyl formed during storage. Such studies have been required previously for temporary tolerance proposals on stored commodities and extensive alteration of the terminal residue in these commodities has been observed.

1b. Additional metabolism studies on a ruminant (preferably a lactating goat) and chickens are needed. These studies should involve feeding of ^{14}C-labeled chorpyrifos-methyl and identification and quantitation of the terminal radioactive residue in meat, milk, poultry and eggs. In all the animal metabolism studies submitted to date, no attempt to identify the nature of the radioactive residue in tissues was made even though radioactive residues in sheep fed labeled chlorpyrifos-methyl ranged up to 11.8 ppm in fat and were also significant in other tissues. No metabolism studies on a lactating ruminant or chickens (eggs) were performed.

2a. Adequate analytical methods for determination of chlorpyrifos-methyl and free 3,5,6-trichloro-2-pyridinol in grains, processing fractions, meat, milk, poultry and eggs are available. This conclusion, however, is tentative until both the questions regarding the metabolism in grains and animals discussed above are resolved and a successful method trial is completed. The method trial of the appropriate procedures will be initiated at such time as all of the other deficiencies in the petition are resolved. Additional methods or modifications of present methods which will allow determination of conjugates or metabolites of chlorpyrifos-methyl in various r.a.c.'s may be needed. Also confirmatory procedures (different glc columns or TLC, etc.) will be needed prior to establishment of tolerances.

2b. In the residue data submitted, identical samples analyzed via the method which determines parent vs determination using the method which measures parent and 3,5,6-trichloro-2-pyridinol as the alcohol always showed much lower residue values when the latter method was used. This question should be addressed. This discrepancy may be due to incompleted hydrolysis of chlorpyrifos-methyl in the grain samples.

3a. The proposed 6 ppm tolerance level for stored grains is appropriate since this is the maximum application rate and combined residues of parent or any metabolites which would occur upon storage could not exceed this value. We consider the high residue value for rice aberrant. This conclusion does not negate, however, our concerns over the nature of the residue discussed above. We will need to know whether conjugates of the pyridinol or perhaps the des-methyl or other compounds would be released from grains containing "aged" residues of chlorpyrifos-methyl when the alkaline hydrolysis described in Method ACR 78.19 is employed. Also, as mentioned above, an explanation of the difference in residue levels found in identical samples of grain analyzed via methods ARC 78.18 and ACR 78.19 should be submitted.

3b. The proposed food-additive tolerances 160 ppm for refined corn oil and 30 ppm for rice milling fractions are appropriate. Based on the processing/milling studies submitted appropriate tolerance levels for other grain by-products are:

Sorghum milling fractions (except flour)	90 ppm
Barley milling fractions (except flour)	90 ppm
Corn soapstock	40 ppm
Oats milling fractions (except flour)	130 ppm
Wheat milling fractions (except flour)	30 ppm

As discussed above, these recommendations are tentative pending satisfactory resolution of questions regarding the nature of the terminal residue in grains and by-products and the adequacy of the proposed methods for determining metabolites and/or conjugates of these compounds which are of concern. Pending the results of the additional studies required, further processing/milling experiments <u>may</u> be needed.

4a. The method used to determine 3,5,6-trichloro-2-pyridinol and chlorpyrifos-methyl both as the alcohol in swine, calves, cows and poultry used a methanol extraction but no hydrolysis step and thus, in all probability did not determine conjugates, if any, of metabolites of chlorpyrifos-methyl. Our concern over the possibility of the formation of conjugates of the pyridinol and other possible metabolites of chlorpyrifos-methyl in animals must be resolved by the additional metabolism studies required above. Depending on the outcome of these metabolism studies, additional feeding studies in livestock employing a hydrolysis step in the method of analysis may be needed.

4b. All of the grains involved in this petition are major feed items which can comprise up to 80 and 70% of the diets of cattle and poultry respectively. Based on the feeding studies submitted, this chemical is classed in Category 2 of Section 180.6(a) with respect to meat, milk, poultry and eggs and therefore tolerances are needed for these

commodities. No estimation of appropriate tolerance levels for these r.a.c.'s can be made, however, until such time as the deficiencies described in 4a above are resolved.

5. The International Tolerance Sheet is attached. The proposed Codex tolerances for milk (0.01 ppm), maize (10 ppm), rice (0.1 ppm) and sorghum (10 ppm) are expressed in terms of parent compound only. Therefore, these tolerances will not be compatible with U.S. tolerance regulations which will include parent plus at the very least, 3,5,6-trichloro-2-pyridinol and perhaps other metabolites. It is also doubtful that the Codex tolerance levels will be compatible with the final U.S. levels.

   At this time, we foresee no mechanism by which the U.S. tolerances could be made compatible with the Codex proposals other those via extensive modification of the Codex tolerances.

Recommendations

We recommend that the proposed tolerances not be established for the reasons given in Conclusions 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b and 5 above. Requirements for resolution of these deficiencies are also discussed in the appropriate conclusions above. The petitioner should be informed of these requirements.

Note to Product Manager: When and if these tolerances are established, the regulation will include at least one metabolite common to both chlorpyrifos and chlorpyrifos-methyl. This metabolite is 3,5,6-trichloro-2-pyridinol. Other common metabilites may also occur. Final determination of what common varieties will be found in the terminal residues of each compound will be made at such time as all of the additional metabolism studies required are submitted. When this determination is made, a paragraph should be added to Section 180.3(d) stipulating where tolerances are established for both chlorpyrifos and chlorpyrifos-methyl on the same raw agricultural commodity the total amount of such residues shall not exceed the highest established tolerance for either pesticide having this (these) metabolite(s).

DETAILED CONSIDERATONS

Formulation

The formulation requested for use is Dow Chemical Reldan 4E Insecticide. This formulation contains 44% technical chlorpyrifos-methyl (4 lb active ingredient/gal). All inerts in the formulation are cleared under Section 180.1001(c). Technical chlorpyrifos-methyl has a 94% minimum purity.

The manufacturing process and impurities in technical chlorpyrifos-methyl are given in Attachment A at the end of this review.

Proposed Use

To control insects in stored grain (barley, corn, oats, rice, sorghum and wheat) chlorpyrifos-methyl is to be applied as a coarse aqueous spray to the moving stream of grain to give a deposit of 3 to 6 ppm of active ingredient on the grain. The higher rate is to be used if the grain is to be stored longer than 6 months.

A grain bin or warehouse use allowing treatment of walls and floors with a 1% solution of chlorpyrifos-methyl is also proposed.

NATURE OF THE RESIDUE

Plant Metabolism

No degradation/metabolism studies on a stored grain have been submitted. Since we have previously required these studies for temporary tolerances on stored grains and since extensive alteration of the terminal residue on these stored commodities has been observed for certain chemicals it is our judgement that a metabolism study on a stored grain is needed. We are especially concerned with the amount and nature of any conjugates formed. The petitioner should be so informed.

Animal Metabolism

Animal metabolism studies on rats and sheep have been submitted. These experiments are discussed below.

The first study utilized ring labeled ^{14}C-chlorpyrifos-methyl and involved a single dosing of two rats with this compound after which the rats were placed in a metabolism cage. The rats had been previously accustomed to the cage. Samples of blood were taken at various intervals up to 72 hours, respired CO_2 was collected as were urine and fecal samples. After 72 hours, the rats were sacrificed and separate tissues were analyzed for ^{14}C equivalents.

Radioactivity on the blood of the rats peaked after approximately 5 hours, within 72 hours some 80-90% of the administered dose was eliminated was respired air, urine or feces. Measurable amounts of radioactivity remained in the tissues after 72 hours.

Analysis of urine via TLC showed 3,5,6-trichloro-2-pyridinol and "origin material" which released irregular amounts of the pyridinol upon rechromatography indicating the possibility of the presence of conjugates of this alcohol. The report did not make clear whether any chlorpyrifos-methyl was observed in the urine. Radioactivity in various tissues ranged from 0.003 ppm in the heart to 0.164 ppm in muscle.

A second rat study using ^{14}C-labeled chlorpyrifos-methyl and whole body autoradiography reflected oral dosing of rats and sampling at 0.5, 1, 3, 5, 7, 24, 72 and 120 hours. The autoradiographs (after 16 days) showed that

radioactivity peaked in tissues at 3 hours post treatment and was undetectable in the body after 120 hours with this technique.

A third metabolism study involved treating ten rats and a sheep with ring-^{14}C-labeled chlorpyrifos-methyl along with administration of ring-^{14}C-labeled 3,5,6-trichloro-2-pyridinol to a second sheep.

The sheep were orally dosed with either chlorpyrifos-methyl or its corresponding pyridinol. Blood, respired CO_2, urine and feces were collected at various intervals. The animals were sacrificed 96 hours after treatment.

The rats were also orally dosed with chlorpyrifos-methyl and urine and feces were collected and the animals were sacrificed after 48 hours.

Metabolites in urine blood and feces were isolated, identified and quantitated using column, glc and thin layer chromatography along with mass spectrometry.

In the urine of the sheep dosed with chlorpyrifos-methyl, the major identified metabolites were the glucuronide of 3,5,6-trichloro-2-pyridinol, and O-methyl-O-(3,5,6-trichloro-2-pyridinol). These metabolites made up 41.6 and 38% of the total radioactivity in the sheep urine. The urine of rats treated with parent compound showed ca. 69% of the glucuronide of 3,5,6-trichloro-2-pyridinol, ca. 18% of the desmethyl metabolite and ca. 14% of free 3,5,6-trichloro-2-pyridinol.

Plasma samples from the sheep treated with chlorpyrifos-methyl showed the same two metabolites as observed in sheep urine in addition to 3,5,6-trichloro-2-pyridinol. No parent was observed in the sheep plasma.

The feces of the sheep treated with parent contained chlorpyrifos-methyl, the des-methyl derivative, and 3,5,6-trichloro-2-pyridinol. Rat feces samples were not analyzed.

It should be noted that rats appear to metabolize chlorpyrifos-methyl much differently than they do the ethyl analog of this compound.

The metabolites in the urine and plasma of the sheep treated with 3,5,6-chloro-2-pyridinol were identified as the alcohol and its glucuronide. Only free pyridinol was obseved in the fecal samples from this sheep.

No radioactivity was detected in the expired air of either species regardless of whether they were fed parent or 3,5,6-trichloro-2-pyridinol.

Radioactivity in various tissues of the sheep fed chlorpyrifos-methyl ranged from 0.23 ppm in the spleen to 11.8 ppm in fat. Radioactive equivalents in the tissues of the sheep fed 3,5,6-trichloro-2-pyridinol ranged from non-detectable in most tissues to 2.17 ppm in the liver. No attempt to identify the radioactivity in sheep tissues was made.

The pyridinol is absorbed into the blood of sheep more rapidly than parent compound. Radioactive equivalents of the pyridinol peaked in the blood of sheep after 8 hours followed by a rapid decrease. Radioactivity in the bloodstream of the sheep fed chlorpyrifos-methyl, however, increased for the first 8 hours then plateaued before slowly decreasing. This phenomena may be due to rapid deployment of parent into fatty tissue followed by a slow release of the hydrolysis product(s) into the bloodstream.

The final three metabolism studies submitted reflected the feeding of ^{14}C-ring-labeled 3,5,6-trichloro-2-pyridinol to rats.

One study involved collection of blood, urine, feces and respired air for up to 72 hours post treatment after which the animals were sacrificed and tissue samples taken.

Radioactivity in blood peaked at 1-3 hours and decreased thereafter. Urine, feces and respired air accounted for ca. 73, 5 and 0.34% of the applied dose after 72 hours. Approximately 2.5% of the dose remained in the tissue after this period. Stress appears to have some effect on the rate of elimination of the pyridinol.

Radioactivity in the urine showed only 3,5,6-trichloro-2-pyridinol and "origin material" after TLC. Radioactivity in various tissues ranged from 0.004 ppm to 0.300 ppm.

The remaining two studies involved a single oral dose of radio-labeled 3,5,6-trichloro-2-pyridinol to rats after which one set of the rats was maintained in metabolism cages until elimination of radioactivity via the urine and feces appeared to be complete. The second group of rats were sacrificed after 24 hours and various tissues were analyzed for radioactivity.

Most of the radioactive dose is excreted in the urine and feces within 48 hours. Radioactive residues in the various tissues ranged from 0.01 ppm in the brain and muscle to 0.17 ppm in the liver after 24 hours.

In summary, the rat studies submitted were inconclusive since no attempt was made to determine the nature of the radioactivity in tissues when either radiolabeled chlorpyrifos-methyl or 3,5,6-trichloro-2-pyridinol were fed to the animals. The pyridinol and conjugates of this compound along with the des-methyl metabolite were observed in urine. A large percentage of the applied radioactivity is eliminated via the urine and feces and little if any of the applied doses is respired as $^{14}CO_2$. The rate of elimination appears to depend on the "stress" the animal undergoes during the experiment. Based on the analysis of urine, the rats also appear to metabolize chlorpyrifos-methyl much differently than the ethyl analog. In all cases, however, measurable amounts of radioactive residues (up to 0.3 ppm) were observed in various tissues after 24 to 72 hours.

The study on sheep indicated that 3,5,6-trichloro-2-pyridinol is absorbed more rapidly than parent compound. No radio-labeled CO_2 was observed in the

respired air of sheep fed either compound. Metabolites observed in sheep urine from the sheep fed chlorpyrifos-methyl contained free 3,5,6-trichloro-2-pyridinol and its glucuronide. Analysis of the plasma of the same sheep showed free pyridinol, the des-methyl metabolite and a glucuronide of the pyridinol. Radioactive residues in feces contained parent compound in addition to the three metabolites found in the plasma above.

No attempt to identify the radioactivity remaining in the tissues of the sheep fed chlorpyrifos-methyl was made even though radioactive residues ranged up to 11.8 ppm in fat.

The sheep fed 3,5,6-trichloro-2-pyridinol showed radioactive pyridinol and its corresponding glucuronide in plasma and urine and only free alcohol in feces. Again no attempt to identify residues in tissues was made. These residues ranged up to 2.17 ppm in the liver.

We do not consider the nature of the residue in animals adequately understood at this time. Additional metabolism studies on a ruminant (preferably a lactating goat), and chickens are needed. These studies should involve feeding of ^{14}C-ring-labeled chlorpyrifos-methyl and identification and quantitation of the various compounds that make up the radioactive residue in ruminant and poultry tissue and milk and eggs. The petitioner should be so informed.

Analytical Methods

Seven methods were submitted in this petition. Two methods measure chlorpyrifos-methyl only in grains, meat, milk, poultry and eggs, three measure 3,5,6-trichloro-2-pyridinol in meat, milk, poultry and eggs and two determine both parent and pyridinol in grains and processing fractions. These seven methods are discussed briefly below. Two other methods which determine chlorpyrifos (diethyl compound) were also submitted. These will not be discussed in this review.

The first method involved extraction with acetone followed by shaking and centrifugation. Chlorpyrifos-methyl is determined via glc using a flame photometric detector. Validation data reflected spiking barley, corn, sorghum, oats, rice and wheat with 1 to 5 ppm of chlorpyrifos-methyl and recoveries ranged from 101 to 112%. Blank crop values were given as 0.00 ppm.

The second method which determined both parent and pyridinol in grains involved extraction with methanol, hydrolysis of chlorpyrifos-methyl to 3,5,6-trichloro-2-pyridinol with NaOH and heat followed by acidification with HCl and two partitionings with toluene. The toluene solution is then treated with N,-O-bis-trimethylsilylacetamide, allowed to stand for 10 minutes at room temperature and an aliquot is then injected into a gas chromatograph equipped with an electron capture detector. Validation data reflected fortification of the grains named in the first method above with 1.5 to 4 ppm of

chlorpyrifos-methyl and 0 to 1.0 ppm of 3,5,6-trichloro-2-pyridinol. Recoveries ranged from 91 to 107%. Blank crop values ranged from 0.02 ppm in wheat and corn to 0.1 ppm in rice.

The next method used glc and a flame photometric detector and determined chlorpyrifos-methyl in muscle, liver, kidney and fat. The method involved extraction with acetone and filtration except for fat where hexane is used for extraction. This is followed by removal of the acetone, addition of hexane and water, separation of the hexane layer and partitioning with acetonitrile. The acetonitrile is evaporated and hexane is added to the sample. The sample is further cleaned-up on a silica gel column using hexane as the eluate, the hexane is removed, the sample is taken up in acetone and injected into the gas chromatograph. Validation data reflected fortification of muscle, liver kidney and fat with 0.01 to 0.5 ppm of chlorpyrifos-methyl and recoveries ranged from 70 to 116% in all tissues. All blank values were given as 0.00 ppm. A modification of this method is used to analyze milk and cream for chlorpyrifos-methyl. The milk or cream sample is heated to 45°C, extracted using methanol: hexane and sodium chloride, centrifuged and then partitioned with acetonitrile as above. Validation data involved spiking milk or cream samples with 0.01 to 1.0 ppm of chlorpyrifos-methyl. Recoveries ranged from 78 to 96%. No blank crop values were given.

The methods which determine 3,5,6-trichloro-2-pyridinol all utilize glc and an electron capture detector. The method for swine tissue involved extraction with methanol, filtration, clean-up on an alumina column, acid: base washing/ partitioning with benzene and derivatization with N,O-bis-trimethylsilyl acetamide prior to injection into the gas chromatograph. Validation data reflected fortification of swine muscle, fat, liver and kidney with 0.01 to 2.0 ppm of 3,5,6-trichloro-2-pyridinol and recoveries ranged from 72 to 99%. Blank values ranged from 0.003 ppm in fat to 0.007 ppm in muscle.

The method for bovine muscle and fat is the same as described for swine tissue above. For bovine, liver and kidney, poultry, eggs and milk and cream slight modifications of this method were employed. The modifications for bovine liver and kidney involved additional acid-base washing/partitioning steps. A different column was employed when poultry fat was analyzed. Milk and cream was heated to 45°C, treated with sodium bicarbonate, washed with benzene, neutralized, then extracted with benzene. The milk and cream samples were cleaned up on an alumina column using the procedures above. Validation data reflected fortification of bovine muscle, fat, liver and kidney at 0.05 to 2 ppm and recoveries ranged from 68% to 104% for all tissues. Blank values ranged from 0.00 to 0.005 ppm. Milk and cream samples were fortified at 0.01 to 1.0 ppm and recoveries ranged from 76 to 100%. No blank values were given.

The other method used to determine 3,5,6-trichloro-2-pyridinol involved blending with H_3PO_4 and benzene, filtration and extraction into $KHCO_3$ solution. This aqueous solution is back extracted with ether then acidified with H_3PO_4 and the residue is then extracted into ether. To the dried ether solution is added acetone and the samples are co-distilled with ethylacetate after deposition on basic alumina. The tube is cooled and the residue is

eluted with aqueous $KHCO_3$. The samples are then concentrated, washed with ether if any fatty substances are observed in the aqueous solution and the solution is acidified with H_3PO_4. The residue is extracted with ether, the ether solution is dried, the ether removed and the samples are derivatized with a solution of N,O-bis-trimethylsilylacetamide and injected into the gas chromatograph. Validation data reflected fortification of beef muscle, poultry, poultry liver and poultry fat at 0.01 to 0.1 ppm and recoveries ranged from 84 to 97% for all tissues. Blank values ranged from 0.000 to 0.041 ppm.

The final method determines chlorpyrifos-methyl and 3, 5, 6-trichloro-2-pyridinol as the alcohol in grains and process fractions via glc using an electron capture detector. The sample is heated (70°C) in alkaline methanolic solution for 1 hour, shaken, centrifuged, acidified and cleaned-up using a prewashed Sep-Pac C_{18} cartridge. The pyridinol is eluted from the cartridge with methanol and aqueous sodium bicarbonate is added to the methanol solution. The sample is washed with benzene, acidified and then extracted into benzene. This solution is treated with N,O-bis-trimethylsilyl acetamide and analyzed via glc. Corn, rice, oats, wheat, barley and sorghum and their processing fractions were fortified with 0.1 to 100 ppm of 3,5,6-trichloro-2-pyridinol and sorghum, rice and barley and their processing fractions were fortified with chlorpyrifos-methyl levels of 0.61 to 61 ppm. Recoveries ranged from 70 to 113 % for the pyridinal and from 75 to 113% for chlorpyrifos-methyl. Only one blank value was given and this was 0.02 ppm.

We tentatively conclude that adequate analytical methods for determination of chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol in grains, processing fractions, meat, milk, poultry and eggs are available pending completion of a successful method trial. No final conclusion can be made, however, until the deficiencies with regards to the metabolism of chlorpyrifos-methyl in grains and animals discussed above are satisfactorily resolved. At such time as these deficiencies are resolved we will initiate a method trial utilizing the appropriate procedures. Also, confirmatory procedures (i.e., use of a different gas chromatopography column, TLC, etc) will be needed before any tolerances could be established. The petitioner should be informed.

Residue Data

Residue data submitted in this petition reflected treatment of barley, corn, oats, sorghum, rice and wheat grains at a rate of 6 ppm with chlorpyrifos-methyl. The grains were then shipped to various locations around the United States and stored for one year. The stored grains were sampled after 0, 1, 3, 6 and 12 months of storage (note: zero days of storage was up to 50 days post-treatment) and analyzed for parent and 3,5,6-trichloro-2-pyridinol.

Barley was stored in Mississippi and California; corn was stored at sites in Michigan, Mississippi, Illinois, and Kansas. Sorghum was stored in Michigan and Texas; oats were stored at sites in Michigan and Kansas; rice was stored in Mississippi, California, Illinois and Texas; and wheat was stored at locations in Michigan, California, Illinois and Kansas.

Residues of chlorpyrifos-methyl immediately after application ranged from 4.3 ppm in corn to 7.0 ppm in rice. When the method which determines chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol as the alcohol was used to analyze these samples, residues of parent were always much lower than above, ranging from 2.6 ppm in corn to 4.6 ppm in rice. This lower value for parent compound when the second method is employed should be addressed in future submissions. It may be due to incomplete hydrolysis of chlorpyrifos-methyl in the initial samples.

Residues in the stored commodity decreased slowly with time with corresponding increases in free pyridinol after about 3 months storage.

Maximum half lives for the various grains in months were 4.3 for barley, 9 for corn, 6.6 for sorghum, 7.6 for oats, 9.8 for rice and 20.5 for wheat. Residues in all grains ranged from 6.5 ppm (in rice) at zero storage time to 0.4 ppm (in barley) after 12 months of storage.

Since 6 ppm is the maximum application rate for stored grains, we consider the high values for rice aberrant, perhaps due to improper application and therefore it is our judgement that regardless of what metabolites are involved, the maximum amount of chlorpyrifos-methyl or metabolites which would occur on the stored grains would be 6 ppm.

We are still concerned, however, over the nature of this residue as discussed in that section above. We also will need to know whether conjugates of the pyridinol or perhaps the des-methyl compound would be released from groups containing aged residues by the alkaline hydrolysis described in method ACR 78.19. That is, does this method detect conjugates of possible chlorpyrifos-methyl metabolites? We would also like some explanation of the difference in residue levels found in identical samples of grain analyzed via methods ACR 78.18 and ACR 78.19. The petitioner should be so informed.

The petitioner has submitted processing studies for each of the grains involved. These studies are discussed below.

Sorghum grain was treated with 6 ppm of chlorpyrifos-methyl stored for 28 days and dry milled. Milling fractions were flour, bran, screenings, shorts and germ. Fractions were analyzed for parent and 3,5,6-trichloro-2-pyridinol. Concentration of residues was observed in bran, shorts, screenings and germ. The greatest concentration (ca. 15X) was observed in screenings. Based on this study we conclude that a food additive tolerance for sorghum milling fractions is needed. The tolerance should be proposed in terms of sorghum milling fractions (except flour). Based on the tolerance for grain and the concentration factor an appropriate level would be 90 ppm (6ppm x 15 = 90). These proposals should be submitted in a revised Section F. The petitioner should be informed.

The next study involved treatment at 6 ppm storage for 29 days and processing of corn grain by solvent extraction. Samples of crude oil, refined oil, bleached oil, soapstock and solvent extracted corn were taken and analyzed for

parent and pyridinol. Concentration of residues was observed in crude and refined oil and soapstock. Concentration factors in refined oil and soapstock were ca. 27x and 6.4x respectively. Food additive tolerances are needed therefore for these two commodities. Based on the tolerance for corn grain and the concentration factors appropriate levels would be 160 ppm for refined corn oil as proposed by the petitioner and 40 for soapstock. These tolerances proposals should be submitted in a revised Secton F. The petitioner should be so informed.

The processing study on oats also involved treatment at 6 ppm, storage for 27 days followed by milling. Concentration of residues was found in hulls (ca. 2.7x), light oats (ca. 2.6x) and dust (ca. 21x). Based on the 6 ppm residue level for oats and the concentration factor we recommended that a food additive tolerance of 130 ppm be proposed for milling fractions of oats (except flour). This should be submitted in a revised Secton F. The petitioner should be informed.

Barley samples were treated with 6 ppm of chlorpyrifos-methyl, stored for 28 days and then malted and made into beer. In all eight fractions were collected: malt, spent grains, filter oil, yeast, malt cleanings, cleaner overs, cleaner thrus and beer. A brief description of the process is submitted. Concentration of residues was observed only in cleaner thrus (ca. 14x). Maximum gross residues in beer were 0.22 ppm using the pyridinol method. Based on this we conclude that a food additive tolerance of 90 ppm in processing (milling) fractions of barley (except flour) would be appropriate. The petitioner should be informed that this proposal should be submitted in a revised Section F.

Wheat grain treated with 6 ppm of chlorpyrifos-methyl was stored for 27 days and then milled and baked. The fractions collected included flour, bran, shorts, reddog, germ and cookies. Concentration of residues was found in bran (ca. 3.6x), shorts (ca. 4.6x), reddog (ca. 2.3) and germ (ca. 4.3x). No concentration was observed in flour or in cookies prepared from the flour. Based on 6 ppm of chlorpyrifos-methyl residues in grain and a maximum concentration of 4.6x in shorts an appropriate food-additive tolerance level for the milling fractions of wheat (except flour) would be 30 ppm. The petitioner should be informed that this proposal should be submitted in a revised Section F.

Two studies for rice processing were submitted. Both reflected treatment of rice with 6 ppm of chlorpyrifos-methyl. In one study the rice was stored for 27 days before milling. In the second study the storage periods were 1 and 14 weeks before milling. Samples of rough rice or grain, hulls, bran, brown rice, white (or milled) rice and grits were taken. In both studies concentration of residues was observed only in the hulls and bran. Concentration factors in the hulls ranged from ca. 3.1 to 5.6x and in bran from ca. 1.5 to 2.4x. Based on the concentration factors and 6.0 ppm residue level on rice grain it is our judgement that the proposed 30 ppm food-additive tolerance level for rice milling fractions is appropriate.

Finally, as discussed above these tolerance recommendations do not alleviate our concerns over the nature of the residue in grain products and by-products or the adequacy of the proposed methods for determining metabolites of concern. The petitioner should be so informed.

Meat, Milk, Poultry and Eggs.

Feeding studies for swine, calves, cows and poultry have been submitted. These are discussed below.

Swine were fed 1,3,30 and 100 ppm of chlorpyrifos-methyl in the diet for 28 days. Animals were slaughtered after the 28 day treatment with the exception of two sets of swine being fed 100 ppm of the compound. These swine were slaughtered after a 7 or 21 day withdrawal period. After slaughter, muscle, liver, kidney and fat samples were analyzed for parent and 3,5,6-trichloro-2-pyridinol. Residues of parent in muscle, liver and kidney tissues of swine fed 1,3 or 30 ppm in the diet ranged from non-detectable to 0.03 ppm. Residues of pyridinol for muscle, liver or kidney tissues of swine fed at the three lower rates ranged from non-detectable to 0.58 ppm. It should be noted here that the method used to determine 3,5,6-trichloro-2-pyridinol used a methanol extraction but no hydrolysis step and thus in all probability did not determine any conjugates of the alcohol. We are concerned over the possibility of the formations of conjugates of this and other possible metabolites of chlorpyrifos-methyl and depending on the outcome of the metabolism studies required above, additional feeding studies may be needed. The petitioner should be so informed. Residues of chlorpyrifos-methyl or 3,5,6-trichloro-2-pyridinol in fat of swine fed 1,3, or 30 ppm of parent in the diet ranged from 0.01 to 0.28 ppm and non-detectable to 0.08 ppm respectively. Swine fed at the 100 ppm level in the diet demonstrated residues of parent in muscle, liver and kidney ranging from 0.01 to 0.17 ppm with the highest values observed in muscle. Residues of chlorpyrifos-methyl in the fat of these swine ranged up to 1.13 ppm of this compound. Residues of parent in all four tissues decreased to non-detectable (0.01 ppm) after seven days withdrawal. With respect to 3,5,6-trichloro-2-pyridinol, residues in muscle and fat of swine fed the 100 ppm of chlorpyrifos-methyl in the diet ranged from 0.19 to 0.34 ppm and decreased to non-detectable after a 7 day withdrawal period. Residues of the pyridinol in liver and kidney of these swine were in the range of 1.2 to 2.3 ppm. These residues also decreased to non-detectable after 7 days of withdrawal.

In all cases above where sample chromatograms were submitted finite peaks were observed for the subject compounds even when the residue levels were considered non-detectable.

The two calf feeding studies submitted reflected feeding of groups of calves of ca. 1, 3, 10, 30 and 100 ppm of chlorpyrifos-methyl for 28 days followed by slaughter and tissue sampling at 0, 7, 14 and 28 days post-treatment. In one study the samples were analyzed only for parent compound and in the second study both chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol were

determined. Again the methods employed in these studies did not utilize a hydrolysis step to release any conjugates of metabolites of chlorpyrifos-methyl.

In both studies residues of chlorpyrifos-methyl in calf muscle and liver were 0.01 ppm at all feeding levels. Residues of parent in calf kidney ranged from 0.01 at the 30 ppm feeding level to 0.95 ppm at the 100 ppm feeding level. Residues of chlorpyrifos-methyl in kidney decreased to 0.01 ppm after 7 days of withdrawal. Residues of parent compound in fat ranged from 0.01 ppm at the 1 ppm feeding level to 0.91 ppm at the 100 pmm level. Residues in fat also decreased to 0.01 ppm after 7 days of withdrawal.

In the second study gross residues of 3,5,6-trichloro-2-pyridinol (including parent) ranged from 0.01 ppm to 0.12 ppm in muscle, from 0.06 to 2.17 ppm in liver, from 0.05 to 1.4 ppm in kidney and from 0.05 to 0.13 ppm in fat at the higher feeding levels. Residues of the pyridinol in liver, kidney and muscle decreased to non-detectable levels after 7 days of withdrawal. No analyses were performed for fat after withdrawal.

A lactating corn feeding study was also submitted. The study utilized 3 cows which were each fed 0, 1, 3, 10 and 100 ppm of chlorpyrifos-methyl in the diet starting with the lowest level and increasing to the next highest level every 14 days. This was then followed by a 14 day withdrawal period. Milk was sampled twice during the first week of each feeding level and three times the second week. Cream was separated and analyzed separately. Both milk and cream were analyzed for chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol. Residues of parent in milk and cream ranged from 0.01 to 0.07 ppm and from 0.07 to 0.50 ppm respectively at the various feeding levels with highest residues observed at the highest dosing level. Residues of chlorpyrifos-methyl in milk decreased to .01 ppm after two days of withdrawal. Residues of 3,5,6-trichloro-2-pyridinol in milk and cream ranged from 0.01 to 0.07 ppm and from 0.01 to 0.07 ppm respectively at the different feeding levels. Highest residues were observed at the highest feeding level. Residues of the pyridinal decreased to 0.01 ppm after a 2 day withdrawal period. As in the earlier feeding studies the methods used to determine chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol did not include a hydrolysis step to release any conjugates of metabolites of chlorpyrifos-methyl in milk.

A chicken feeding study was also submitted. This study reflected feeding chickens 1, 3, 10, 30 and 100 ppm of chlorpyrifos-methyl in the diet for 28 days. Eggs were sampled periodically and tissues were taken at 0, 7, and 14 days past medication. Residues of parent in muscle and liver were 0.01 to 0.01 ppm while residues of this compound in fat and eggs ranged from 0.01 to 0.1 ppm and from 0.01 to 0.03 ppm respectively. Residues of 3,5,6-trichloro-2-pyridinol were 0.05 ppm in muscle and fat and ranged from 0.05 to 0.12 ppm in liver and from 0.05 to 0.08 ppm in eggs. Residues of parent decreased to 0.01 ppm in all tissues and eggs after 6 to 7 days of withdrawal. Residues of the pyridinol also decreased to 0.05 ppm after the 6 to 7 day withdrawal period with the exception of one sample of liver which was

still 0.06 ppm 7 days past medication. Single egg samples showed residues of parent and alcohol of 0. 1 to 0.03 ppm and 0.05 to 0.07 ppm respectively. An egg sample separated into yolk and whites showed that most of the residue was located in the yolk.

As in previous feeding studies no hydrolysis step intended to release any conjugates of chlorpyrifos-methyl metabolites was employed in the method used for determination of residues in poultry tissue and eggs.

All of the subject grains of this petition are major feed items which comprise up to 80 and 70% of the diets of cattle and poultry respectively. Based on the feeding studies discussed above we class this chemical in category 2 of Section 180.6(a) with respect to meat, milk, poultry and eggs. We cannot however recommend appropriate tolerance levels for these commodities until such time as the deficiencies in animals and plant metabolism and our concern over the lack of a hydrolysis step for release of any conjugates of chlorpyrifos-methyl metabolites in the feeding studies are resolved. Additional feeding studies employing a hydrolysis step in the method of analysis may be needed. The petitioner should be so informed.

Other Considerations

The International Tolerance Sheet is attached. There are proposed Codex tolerances for milk (0.01 ppm), maize (10 ppm), rice (0.1 ppm) and sorghum (10 ppm). The residue of concern in these proposals is parent compound only. These tolerances are not compatible with present proposals or with our tentatively recommended tolerance regulations and levels. At this time we foresee no mechanism by which the U.S. tolerances could be made compatible with the Codex proposals other than via extensive modification of the Codex tolerances.

Note to Product Manager: When and if these tolerance are established the regulation will include at least one metabolite common to both chlorpyrifos and chlorpyrifos-methyl. This metabolite is 3,5,6-trichloro-2-pyridinol. Other common metabolites may also occur. Final determination of what common moieties will be found in the terminal residues of each compound will be made at such time as all of the additional metabolism studies required are submitted. When this determination is made a paragraph should be added to 180.3(d) stipulating that where tolerances are established for both chlorpyrifos and chlorpyrifos-methyl on the same raw agricultural commodity the total amount of such residues shall not exceed the highest established tolerance for either pesticide having this (these) metabolite(s). The petitioner should be so informed.

TS-769:RCB:R.B.Perfetti, Raven:CM#2:RM 810:March 5, 1981

CC: RF, Circ., Perfetti, Watts, FDA, TOX, EEB, EFB, PP#0F2423 and FAP#0H5277

RDI: Quick, 2/27/81; Schmitt, 3/2/81

INTERNATIONAL RESIDUE LIMIT STATUS

CHEMICAL Chlorpyrifos-methyl (Reldan) PETITION NO PP#0F2423/FAP#0H5277
(0,0-dimethyl 0-(3,5,6-trichloro-2-pyridyl)phosphorothioate)

CCPR NO. 90

Code Status Step 8

[ ] No Codex Proposal
[ ] Step 6 or above

Proposed U. S. Tolerances

Chlorpyrifos-methyl and its metabolite 3,5,6-trichloro-2-pyridinol

Residue (if Step 9):

Chloropyrifos-methyl

Crop(s)	Limit (mg/kg)
Milk	0.01 mg/kg
Maize	10 mg/kg
Rice	0.1 mg/kg
Sorghum	10 mg/kg

Residue:

Crop(s)	Tol. (ppm)
milk fat	0.1
milk (whole)	0.02
poultry	0.05
eggs	0.05
corn grain	-6.0
barley	-6.0
oats	-6.0
rice	-6.0
sorghum	6.0
wheat	6.0
Cattle, goats and sheep meat	.1
Cattle, goats and sheep fat	0.2
Cattle, goats and sheep meat by products	1.0
Horses and hogs meat	0.1
Horses and hogs fat	0.3
Horses and hogs meat by-products	0.5

Food Additive Tolerances

Corn oil	160
Milling fractions (except flour) of barley, corn, oats, sorghum and wheat	20
Milling fractions of rice	30

CANADIAN LIMIT

Residue: Chloropyrifos-methyl

Crop	Limit (ppm)
None an these commodities	

MEXICAN TOLERANCIA

Residue: None

Crop	Tolerancia (ppm)
None	

Notes

Manufacturing process information may be entitled to confidential treatment

ATTACHMENT A

Chlorpyrifos-methyl is manufactured [redacted]

Impurities in the technical material include (Note: Maximum amounts of impurity are given in parentheses): [redacted]

We foresee no residue problems with the small amounts of impurities found in the formulation.

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Chemical:	**Chlorpyrifos-methyl (ANSI)**
PC Code:	**059102**
HED File Code	**11000 Chemistry Reviews**
Memo Date:	**03/13/1981**
File ID:	**00000000**
Accession Number:	**412-01-0166**

HED Records Reference Center
05/23/2001